The above-mentioned sandstones, instead of "being overlain to the west by the light-colored sandstones of the Ione formation," are in reality stratigraphically higher. These sandstones have been worn away from most of this area and only a few residuals remain.

After this great erosion, andesitic tuffs and tuff breccias covered all. During the Pleistocene and Recent time much of the andesitic material has been removed re-exposing the older rocks beneath.

The Ione has been repeatedly correlated with the Auriferous gravels of the Sierras and the upper portion with the rhyolitic tuffs. It can no longer be doubted that the Ione is of the same age as the Rhyolitic tuff and the Auriferous gravels, and since the Ione is clearly Tejon-Eocene, the Auriferous gravels, their correlative, must be upper Eocene, at least in part and the land equivalent of the marine Tejon.

Roy E. Dickerson

The Increase in Permeability of the Frog's Egg at the Beginning of Development and the Preservation of the Life of the Egg [1]

Three years ago, it was observed that the unfertilized frog's egg could be made parthenogenetic by a momentary electric shock, and reasons given for supposing that the electric shock (or the spermatozoon in normal fertilization) increased the permeability of the egg.[2] Recently, I proved this supposition to be correct. The permeability of the unfertilized egg to NaCl was found to have increased on stimulating the egg with an electric shock (which caused it to begin normal development).

Several methods were tried for the quantitative estimation of sodium ions, but the results with such small quantities would not be considered trustworthy had they not tallied with the more certain results on the determination of chlorine ions with the nephelometer, and only the latter will be described here. The technique was as follows:

A "pregnant" female of *Rana pipiens* was washed in alcohol and then in water, pithed and opened. The eggs were removed from the oviducts without mechanical injury or contamination with blood or lymph. These eggs were washed 10 minutes in a large volume of H_2O[3] and divided into two exactly equal masses. Each mass was placed in 30 c.c. of H_2O and allowed to remain for 30 minutes while the jelly swelled. The water that had not been taken up by the jelly was analyzed and the Na+ and Cl— found to be the same for both lots. Then lot 1 was stimulated by an electric shock from clean platinum electrodes[4] and lot 2 used as a control. 20 c.c. of H_2O were added to each lot and at the end of one hour this water was analyzed. There was more Na+ and Cl— in the water from the stimulated eggs than the control, the ratio of Cl— being 10 to 7. This is a very small difference, but it must be remembered that the salt in diffusing out of the egg is held for some time by the "fertilization membrane" and the thick jelly surrounding the egg. Consequently 30 c.c. of H_2O were added to each lot and allowed to remain eight hours to give time for the salts to diffuse through the jelly. There was now found three times as much Cl— that had diffused out of the stimulated eggs as had diffused out of the control. Whether this increase in permeability is the *cause* of development has not been determined, but it is not restricted to the frog's egg, since I found the same true of the sea urchins' egg,[5] a fact which has been confirmed by Gray[6] at Plymouth.

The unfertilized frog's egg placed in fresh or distilled water continues to swell until death ensues. This death is probably caused by the swelling, and the latter by the osmotic pressure of the soluble substances contained within

[1] Preliminary note.

[2] McClendon, Science, N. S., Vol. 33, p. 629.

[3] H_2O means water redistilled in quartz.

[4] In about one minute all of the eggs had turned the black pole upward; 3 hours later the first cleavage began.

[5] McClendon, *Amer. Jour. Physiol.*, 1910, Vol. 27, p. 240.

[6] Gray, *Jour. Marine Biol. Assn. U. K.*, 1913, Vol. 10, p. 50.

the egg. The increased permeability allows the escape of NaCl and lowers the internal osmotic pressure, thus retarding the swelling and preserving the life of the egg.

The decreased swelling of the developing egg can easily be measured. Forty-six eggs were removed from the oviduct and 23 placed on the bottom of a dry glass dish and 23 in a similar one. They were covered with distilled water and the first lot stimulated with an electric shock[7] and the second lot used as a control. The longest and shortest diameter of each egg was measured and the mean of all of each lot determined. The mean diameter of the eggs of the first lot on an average of 30 minutes after stimulation was 1.47 mm., whereas the mean diameter of the control was 1.52 mm. This is in confirmation of the results of Biataszewicz and of Bachmann. Biataszewicz[8] says that the frog's egg momentarily shrinks immediately after fertilization, due to fluid passing out of the egg into the perivitelline space. This is probably due to the increase in permeability. The quantity of fluid in the perivitelline space immediately after fertilization is too small to be collected, but it accumulates during development due to absorption of water from the medium and finally can be removed with a very fine thin-walled capillary pipette. Bachmann thinks that the osmotic substances in this fluid are secreted by the suckers, but the fluid is more abundant in *Amblystoma,* which has no suckers. I found it to contain relatively large quantities of NaCl, considering the fact that the "fertilization membrane" is permeable to NaCl. In *Amblystoma* this fluid is in such abundance that one might hope to make a complete analysis. I found it to contain besides water and NaCl, an organic substance which greatly reduced the surface tension. A very slight Millon's reaction was obtained after evaporating the solution down to dryness. Although the perivitelline space is larger in eggs in distilled water than in tap water, after the space has once enlarged, it is not readily shrunken by salts in the medium. The diameter of the "fertilization membrane" of an egg taken from distilled water was 13 mm. It was placed in Ringer's solution (for mammals) and in two days it had decreased only to 11.5 mm.

Bachmann and Runnström[9] found that the osmotic pressure of the frog's egg dropped enormously on fertilization and they do not believe that this can be accounted for by loss of salt. They seem to consider the egg as a diphasic system in which the watery phase forms the main bulk of the egg. On the contrary, the frog's egg is a four-phase system in which the watery phase is a very small fraction of the total volume. The bulkiest phase consists of yolk platelets composed of lecithalbumin swollen with water. The oil droplets are small and pigment granules smaller. It seems probable that the watery phase, which I found to contain 85 per cent. water and which fills the interstices between the other bodies, would freeze first in freezing point determinations, and we may assume that Bachmann and Runnström determined the Δ and calculated the osmotic pressure of this phase. Since the watery phase is but a small fraction of the volume of the entire egg, the loss of only a minute quantity of NaCl would be necessary in order to greatly lower the osmotic pressure. It should also be noted that Bachmann and Runnström did not remove all of the jelly from the eggs before crushing and freezing them and, consequently, the calculated osmotic pressure for the fertilized eggs is probably too low. The unfertilized eggs which they used were taken from the ovary and were not surrounded by jelly.

Bachmann and Runnström suppose the reduction of osmotic pressure of the frog's egg on fertilization to be due to the adsorption of salts to the proteins, following a sort of "coagulation" of the proteins. If it is true that the salts are adsorbed after "coagulation" by fertilization, we might suppose that they would be adsorbed after coagulation by heat, which could be tested by experiment. 564

[7] The first lot rotated normally and, 3 hours later, began the first cleavage.

[8] *Bull. Acad. Sc. Cracow Math.-Nat.,* October, 1908.

[9] *Biochem. Zeitschr.,* 1909, Vol. 22, p. 390.

grams (about 50 c.c.) of ripe ovarian eggs of *Rana pipiens* were boiled in absolute alcohol and extracted with absolute ether and dried at 135°. They were then powdered and boiled in 200 c.c. distilled water slightly acidulated with acetic acid (free from salts) to coagulate the proteins, and filtered. The filtrate was evaporated down and both filtrate and precipitate charred and extracted and titrated for chlorides. The filtrate required 1.55 c.c. 1/10 normal $AgNO_3$, whereas the precipitate required but .2 c.c., which might be due to the small amount of filtrate held in the precipitate. It thus appears that very little if any salt was adsorbed. If all this chloride is NaCl it would make a .00756 molecular solution of the same volume as the egg. However, the osmotic pressure of the ovarian egg corresponds to that of a .166 normal NaCl solution. If this osmotic pressure is due chiefly to NaCl it must be confined to the watery phase which must equal .0455 or about 1/20 of the volume of the egg.

I found that frog's eggs lose NaCl continuously during their development in distilled water, hence they must be permeable to NaCl for some time after fertilization. This is in harmony with the fact that pure NaCl solutions are not so toxic to the frog's egg as to the eggs of many other animals. I found that those salt solutions which were toxic to fish eggs increased the permeability, but the fertilized frog's egg is already permeable.[10] Some of the older work on the effect of pure NaCl on the frog's egg might be objected to on the ground that the NaCl solution became contaminated by Ca contained in the egg jelly. Therefore I made a series of experiments in which small numbers of frog's eggs were washed for an hour in several liters of distilled water, and placed in several liters of pure $NaNO_3$ solution. Very dilute solutions were non-toxic. One tenth molecular solutions showed a toxic effect in 48 hours, but this may have been due to osmotic pressure, since the addition of 1.6 c.c. of a molecular $CaCl_2$ solution to the liter did not decrease the toxicity. The toxicity of all salts is not due entirely to osmotic pressure, since I found lithium salts to be slightly more toxic than sodium salts of same osmotic pressure.

All of the abnormalities in the lesser toxic salt solutions which I have observed or found in the literature, are characterized by a retardation or failure of the white pole to segment. This is also true of abnormalities produced by centrifugal force or other mechanical agents applied to the unsegmented egg. This unsegmented white pole prevents or retards the downgrowth of the black cell layer, and in extreme cases leads to the so-called "lithium larvæ." These embryos may regenerate and become normal tadpoles. The more toxic solutions prevent segmentation of the white pole and cause swelling of serous cavities (pericardium) and a separation or loosening up of the black cells, accompanied by death of some of these cells (a condition called by Roux "framboisea"). This condition (also seen in fish embryos) occurs after the frog's embryo has partially regained its semipermeability, and may be due to an abnormal increase in permeability by the salt solution.

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June 1, 1914

[10] Although it is more permeable to water than to salts.

THE AMERICAN CHEMICAL SOCIETY. III

Division of Physical and Inorganic Chemistry

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Rapid Detection of Arsenic in Poison Cases by the Marsh Test: James R. Withrow.

It seems to have been the experience for a long time that the number of cases where arsenic is the poison used exceeds that of all other poisons combined. Certain and rapid detection is therefore a matter of much moment. Any effort to make old methods more certain and to eliminate possibility of error by contamination or to abbreviate, thus reducing opportunity for loss, are desirable. The Berzelius-Liebig modification of the Marsh test (1836) has long enjoyed confidence as one of most satisfactory tests. It requires for universal certainty of results the elimination of organic